

## **RAW SEQUENCE LISTING**

**The Biotechnology Systems Branch of the Scientific and Technical  
Information Center (STIC) no errors detected.**

Application Serial Number: 10/031, 496E  
Source: JFW16  
Date Processed by STIC: 03/22/2007

# ***ENTERED***



IFW16

## RAW SEQUENCE LISTING

DATE: 03/22/2007

PATENT APPLICATION: US/10/031,496E

TIME: 12:58:50

Input Set : N:\efs\03\_22\_07\10031496e\_efs\99-45\_SEQUENCE\_ST25.txt

Output Set: N:\CRF4\03222007\J031496E.raw

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3 <110> APPLICANT: National Renewable Energy Laboratory (NREL)
5 <120> TITLE OF INVENTION: Cellobiohydrolase I Gene and Improved Variants
7 <130> FILE REFERENCE: NREL 99-45
9 <140> CURRENT APPLICATION NUMBER: 10/031,496E
10 <141> CURRENT FILING DATE: 2002-01-14
12 <160> NUMBER OF SEQ ID NOS: 97
14 <170> SOFTWARE: PatentIn version 3.4
16 <210> SEQ ID NO: 1
17 <211> LENGTH: 45
18 <212> TYPE: DNA
19 <213> ORGANISM: Artificial
21 <220> FEATURE:
22 <223> OTHER INFORMATION: Nucleotide encoding linker
24 <400> SEQUENCE: 1
25 cctcccggcg gaaacccgcc tggcaccacc accacccgcc gccca 45
28 <210> SEQ ID NO: 2
29 <211> LENGTH: 15
30 <212> TYPE: PRT
31 <213> ORGANISM: Trichoderma reesei
33 <400> SEQUENCE: 2
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36 1 5 10 15
39 <210> SEQ ID NO: 3
40 <211> LENGTH: 24
41 <212> TYPE: DNA
42 <213> ORGANISM: Artificial sequence
44 <220> FEATURE:
45 <223> OTHER INFORMATION: Nucleotide encoding linker
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48 ggcggaaacc cgcctggcac cacc 24
51 <210> SEQ ID NO: 4
52 <211> LENGTH: 1551
53 <212> TYPE: DNA
54 <213> ORGANISM: Trichoderma reesei
56 <400> SEQUENCE: 4
57 atgtatcgga agttggccgt catctcggcc ttcttggcca cagctcgtgc tcagtcggcc 60
59 tgcactctcc aatcggagac tcacccgcct ctgacatggc agaaatgctc gtctggtggc 120
61 acgtgcactc aacagacagg ctccgtggtc atcgacgcca actggcgctg gactcacgct 180
63 acgaacagca gcacgaactg ctacgatggc aacacttgga gctcgaccct atgtcctgac 240
65 aacgagacct gcgcgaagaa ctgctgtctg gacggtgccg cctacgcgtc cacgtacgga 300
67 gttaccacga gcggtaacag cctctccatt ggctttgtca cccagtctgc gcagaagaac 360
69 gttggcgctc gcctttacct tatggcgagc gacacgacct accaggaatt caccctgctt 420
71 ggcaacgagt tctctttcga tgttgatggt tcgcagctgc cgtgcggtt gaacggagct 480

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73 ctctacttcg tgtccatgga cgcggatggt ggcgtgagca agtatccac caacaccgct 540
75 ggcgccaagt acggcacggg gtactgtgac agccagtgtc cccgcgatct gaagttcatc 600
77 aatggccagg ccaacgttga gggctgggag ccgtcatcca acaacgcgaa cacgggcatt 660
79 ggaggacacg gaagctgctg ctctgagatg gatattctggg aggccaactc catctccgag 720
81 gctcttacc cccacccttg cactgactgtc ggccaggaga tctgcgaggg tgatgggtgc 780
83 ggcggaactt actccgataa cagatatggc ggcaacttgc atcccgatgg ctgcgactgg 840
85 aaccataacc gctggggcaa caccagcttc tacggccctg gctcaagctt taccctcgat 900
87 accaccaaga aattgaccgt tgtcaccag ttcgagacgt cgggtgccat caaccgatac 960
89 tatgtccaga atggcgtcac tttccagcag cccaacgccg agcttggtag ttactctggc 1020
91 aacgagctca acgatgatta ctgcacagct gaggaggcag aattcggcgg atcctctttc 1080
93 tcagacaagg gcggcctgac tcagtccaag aaggctacct ctggcggcat ggttctggtc 1140
95 atgagtctgt gggatgatta ctacgccaac atgtgtgtggc tggactccac ctaccgcaca 1200
97 aacgagacct cctccacacc cggtgccgtg cgcggaagct gctccaccag ctccggtgtc 1260
99 cctgctcagg tcgaattcca gtctcccaac gccaaaggta ctttctccaa catcaagttc 1320
101 ggacccattg gcagaccgg caaccctagc ggcggcaacc ctcccggcgg aaaccgcctt 1380
103 ggcaccacca ccaccggccg cccagccact accactggaa gctctcccg acctaccag 1440
105 tctcactacg gccagtgcgg cggatttggc tacagcggcc ccacggtctg cgccagcggc 1500
107 acaacttgcc aggtcctgaa cccttactac tctcagtgcc tgtaaagctc c 1551

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110 &lt;210&gt; SEQ ID NO: 5

111 &lt;211&gt; LENGTH: 514

112 &lt;212&gt; TYPE: PRT

113 &lt;213&gt; ORGANISM: Trichoderma reesei

115 &lt;400&gt; SEQUENCE: 5

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117 Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr Ala Arg
118 1 5 10 15
121 Ala Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr
122 20 25 30
125 Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser
126 35 40 45
129 Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
130 50 55 60
133 Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
134 65 70 75 80
137 Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala
138 85 90 95
141 Ser Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe
142 100 105 110
145 Val Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met
146 115 120 125
149 Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe
150 130 135 140
153 Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala
154 145 150 155 160
157 Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro
158 165 170 175
161 Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln
162 180 185 190
165 Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
166 195 200 205

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169 Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly
170      210                      215                      220
173 Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu
174 225                      230                      235                      240
177 Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu
178                      245                      250                      255
181 Gly Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr
182                      260                      265                      270
185 Cys Asp Pro Asp Gly Cys Asp Trp Asn Pro Tyr Arg Leu Gly Asn Thr
186                      275                      280                      285
189 Ser Phe Tyr Gly Pro Gly Ser Ser Phe Thr Leu Asp Thr Thr Lys Lys
190      290                      295                      300
193 Leu Thr Val Val Thr Gln Phe Glu Thr Ser Gly Ala Ile Asn Arg Tyr
194 305                      310                      315                      320
197 Tyr Val Gln Asn Gly Val Thr Phe Gln Gln Pro Asn Ala Glu Leu Gly
198                      325                      330                      335
201 Ser Tyr Ser Gly Asn Glu Leu Asn Asp Asp Tyr Cys Thr Ala Glu Glu
202                      340                      345                      350
205 Ala Glu Phe Gly Gly Ser Ser Phe Ser Asp Lys Gly Gly Leu Thr Gln
206                      355                      360                      365
209 Phe Lys Lys Ala Thr Ser Gly Gly Met Val Leu Val Met Ser Leu Trp
210      370                      375                      380
213 Asp Asp Tyr Tyr Ala Asn Met Leu Trp Leu Asp Ser Thr Tyr Pro Thr
214 385                      390                      395                      400
217 Asn Glu Thr Ser Ser Thr Pro Gly Ala Val Arg Gly Ser Cys Ser Thr
218                      405                      410                      415
221 Ser Ser Gly Val Pro Ala Gln Val Glu Ser Gln Ser Pro Asn Ala Lys
222      420                      425                      430
225 Val Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn
226      435                      440                      445
229 Pro Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr
230      450                      455                      460
233 Thr Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln
234 465                      470                      475                      480
237 Ser His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val
238                      485                      490                      495
241 Cys Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln
242      500                      505                      510
245 Cys Leu
249 <210> SEQ ID NO: 6
250 <211> LENGTH: 514
251 <212> TYPE: PRT
252 <213> ORGANISM: Trichoderma reesei CBH1-N45A
254 <400> SEQUENCE: 6
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257 1      5      10      15
260 Ala Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr
261      20      25      30
264 Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser

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|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |  |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|--|--|
| 265 |     | 35  |     | 40  |     | 45  |     |     |     |     |     |     |     |     |     |     |  |  |  |
| 268 | Val | Val | Ile | Asp | Ala | Asn | Trp | Arg | Trp | Thr | His | Ala | Thr | Ala | Ser | Ser |  |  |  |
| 269 |     | 50  |     |     |     | 55  |     |     |     |     |     | 60  |     |     |     |     |  |  |  |
| 272 | Thr | Asn | Cys | Tyr | Asp | Gly | Asn | Thr | Trp | Ser | Ser | Thr | Leu | Cys | Pro | Asp |  |  |  |
| 273 | 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |  |  |  |
| 276 | Asn | Glu | Thr | Cys | Ala | Lys | Asn | Cys | Cys | Leu | Asp | Gly | Ala | Ala | Tyr | Ala |  |  |  |
| 277 |     |     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |  |  |  |
| 280 | Ser | Thr | Tyr | Gly | Val | Thr | Thr | Ser | Gly | Asn | Ser | Leu | Ser | Ile | Gly | Phe |  |  |  |
| 281 |     |     |     | 100 |     |     |     |     | 105 |     |     |     |     | 110 |     |     |  |  |  |
| 284 | Val | Thr | Gln | Ser | Ala | Gln | Lys | Asn | Val | Gly | Ala | Arg | Leu | Tyr | Leu | Met |  |  |  |
| 285 |     |     | 115 |     |     |     |     | 120 |     |     |     |     | 125 |     |     |     |  |  |  |
| 288 | Ala | Ser | Asp | Thr | Thr | Tyr | Gln | Glu | Phe | Thr | Leu | Leu | Gly | Asn | Glu | Phe |  |  |  |
| 289 |     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |  |  |  |
| 292 | Ser | Phe | Asp | Val | Asp | Val | Ser | Gln | Leu | Pro | Cys | Gly | Leu | Asn | Gly | Ala |  |  |  |
| 293 | 145 |     |     |     |     | 150 |     |     |     |     | 155 |     |     |     | 160 |     |  |  |  |
| 296 | Leu | Tyr | Phe | Val | Ser | Met | Asp | Ala | Asp | Gly | Gly | Val | Ser | Lys | Tyr | Pro |  |  |  |
| 297 |     |     |     |     | 165 |     |     |     |     | 170 |     |     |     |     | 175 |     |  |  |  |
| 300 | Thr | Asn | Thr | Ala | Gly | Ala | Lys | Tyr | Gly | Thr | Gly | Tyr | Cys | Asp | Ser | Gln |  |  |  |
| 301 |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |     |  |  |  |
| 304 | Cys | Pro | Arg | Asp | Leu | Lys | Phe | Ile | Asn | Gly | Gln | Ala | Asn | Val | Glu | Gly |  |  |  |
| 305 |     |     | 195 |     |     |     |     | 200 |     |     |     |     | 205 |     |     |     |  |  |  |
| 308 | Trp | Glu | Pro | Ser | Ser | Asn | Asn | Ala | Asn | Thr | Gly | Ile | Gly | Gly | His | Gly |  |  |  |
| 309 |     | 210 |     |     |     | 215 |     |     |     |     |     | 220 |     |     |     |     |  |  |  |
| 312 | Ser | Cys | Cys | Ser | Glu | Met | Asp | Ile | Trp | Glu | Ala | Asn | Ser | Ile | Ser | Glu |  |  |  |
| 313 | 225 |     |     |     |     | 230 |     |     |     |     | 235 |     |     |     | 240 |     |  |  |  |
| 316 | Ala | Leu | Thr | Pro | His | Pro | Cys | Thr | Thr | Val | Gly | Gln | Glu | Ile | Cys | Glu |  |  |  |
| 317 |     |     |     |     | 245 |     |     |     |     | 250 |     |     |     |     | 255 |     |  |  |  |
| 320 | Gly | Asp | Gly | Cys | Gly | Gly | Thr | Tyr | Ser | Asp | Asn | Arg | Tyr | Gly | Gly | Thr |  |  |  |
| 321 |     |     |     | 260 |     |     |     |     | 265 |     |     |     |     | 270 |     |     |  |  |  |
| 324 | Cys | Asp | Pro | Asp | Gly | Cys | Asp | Trp | Asn | Pro | Tyr | Arg | Leu | Gly | Asn | Thr |  |  |  |
| 325 |     |     | 275 |     |     |     |     | 280 |     |     |     |     | 285 |     |     |     |  |  |  |
| 328 | Ser | Phe | Tyr | Gly | Pro | Gly | Ser | Ser | Phe | Thr | Leu | Asp | Thr | Thr | Lys | Lys |  |  |  |
| 329 |     | 290 |     |     |     |     | 295 |     |     |     |     | 300 |     |     |     |     |  |  |  |
| 332 | Leu | Thr | Val | Val | Thr | Gln | Phe | Glu | Thr | Ser | Gly | Ala | Ile | Asn | Arg | Tyr |  |  |  |
| 333 | 305 |     |     |     |     | 310 |     |     |     |     | 315 |     |     |     | 320 |     |  |  |  |
| 336 | Tyr | Val | Gln | Asn | Gly | Val | Thr | Phe | Gln | Gln | Pro | Asn | Ala | Glu | Leu | Gly |  |  |  |
| 337 |     |     |     | 325 |     |     |     |     |     | 330 |     |     |     | 335 |     |     |  |  |  |
| 340 | Ser | Tyr | Ser | Gly | Asn | Glu | Leu | Asn | Asp | Asp | Tyr | Cys | Thr | Ala | Glu | Glu |  |  |  |
| 341 |     |     |     | 340 |     |     |     |     | 345 |     |     |     |     | 350 |     |     |  |  |  |
| 344 | Ala | Glu | Phe | Gly | Gly | Ser | Ser | Phe | Ser | Asp | Lys | Gly | Gly | Leu | Thr | Gln |  |  |  |
| 345 |     |     | 355 |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |  |  |  |
| 348 | Phe | Lys | Lys | Ala | Thr | Ser | Gly | Gly | Met | Val | Leu | Val | Met | Ser | Leu | Trp |  |  |  |
| 349 |     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |  |  |  |
| 352 | Asp | Asp | Tyr | Tyr | Ala | Asn | Met | Leu | Trp | Leu | Asp | Ser | Thr | Tyr | Pro | Thr |  |  |  |
| 353 | 385 |     |     |     |     | 390 |     |     |     |     | 395 |     |     |     | 400 |     |  |  |  |
| 356 | Asn | Glu | Thr | Ser | Ser | Thr | Pro | Gly | Ala | Val | Arg | Gly | Ser | Cys | Ser | Thr |  |  |  |
| 357 |     |     |     |     | 405 |     |     |     |     | 410 |     |     |     | 415 |     |     |  |  |  |
| 360 | Ser | Ser | Gly | Val | Pro | Ala | Gln | Val | Glu | Ser | Gln | Ser | Pro | Asn | Ala | Lys |  |  |  |
| 361 |     |     |     | 420 |     |     |     |     | 425 |     |     |     |     | 430 |     |     |  |  |  |

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364 Val Thr Phe Ser Asn Ile Lys Phe Gly Pro Ile Gly Ser Thr Gly Asn
365          435          440          445
368 Pro Ser Gly Gly Asn Pro Pro Gly Gly Asn Pro Pro Gly Thr Thr Thr
369          450          455          460
372 Thr Arg Arg Pro Ala Thr Thr Thr Gly Ser Ser Pro Gly Pro Thr Gln
373 465          470          475          480
376 Ser His Tyr Gly Gln Cys Gly Gly Ile Gly Tyr Ser Gly Pro Thr Val
377          485          490          495
380 Cys Ala Ser Gly Thr Thr Cys Gln Val Leu Asn Pro Tyr Tyr Ser Gln
381          500          505          510
384 Cys Leu
388 <210> SEQ ID NO: 7
389 <211> LENGTH: 514
390 <212> TYPE: PRT
391 <213> ORGANISM: Trichoderma reesei CBH1-N270A
393 <400> SEQUENCE: 7
395 Met Tyr Arg Lys Leu Ala Val Ile Ser Ala Phe Leu Ala Thr Ala Arg
396 1          5          10          15
399 Ala Gln Ser Ala Cys Thr Leu Gln Ser Glu Thr His Pro Pro Leu Thr
400          20          25          30
403 Trp Gln Lys Cys Ser Ser Gly Gly Thr Cys Thr Gln Gln Thr Gly Ser
404          35          40          45
407 Val Val Ile Asp Ala Asn Trp Arg Trp Thr His Ala Thr Asn Ser Ser
408          50          55          60
411 Thr Asn Cys Tyr Asp Gly Asn Thr Trp Ser Ser Thr Leu Cys Pro Asp
412 65          70          75          80
415 Asn Glu Thr Cys Ala Lys Asn Cys Cys Leu Asp Gly Ala Ala Tyr Ala
416          85          90          95
419 Ser Thr Tyr Gly Val Thr Thr Ser Gly Asn Ser Leu Ser Ile Gly Phe
420          100          105          110
423 Val Thr Gln Ser Ala Gln Lys Asn Val Gly Ala Arg Leu Tyr Leu Met
424          115          120          125
427 Ala Ser Asp Thr Thr Tyr Gln Glu Phe Thr Leu Leu Gly Asn Glu Phe
428          130          135          140
431 Ser Phe Asp Val Asp Val Ser Gln Leu Pro Cys Gly Leu Asn Gly Ala
432 145          150          155          160
435 Leu Tyr Phe Val Ser Met Asp Ala Asp Gly Gly Val Ser Lys Tyr Pro
436          165          170          175
439 Thr Asn Thr Ala Gly Ala Lys Tyr Gly Thr Gly Tyr Cys Asp Ser Gln
440          180          185          190
443 Cys Pro Arg Asp Leu Lys Phe Ile Asn Gly Gln Ala Asn Val Glu Gly
444          195          200          205
447 Trp Glu Pro Ser Ser Asn Asn Ala Asn Thr Gly Ile Gly Gly His Gly
448          210          215          220
451 Ser Cys Cys Ser Glu Met Asp Ile Trp Glu Ala Asn Ser Ile Ser Glu
452 225          230          235          240
455 Ala Leu Thr Pro His Pro Cys Thr Thr Val Gly Gln Glu Ile Cys Glu
456          245          250          255
459 Gly Asp Gly Cys Gly Gly Thr Tyr Ser Asp Asn Arg Tyr Gly Gly Thr

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RAW SEQUENCE LISTING ERROR SUMMARY      DATE: 03/22/2007  
PATENT APPLICATION:    US/10/031,496E      TIME: 12:58:51

Input Set : N:\efs\03\_22\_07\10031496e\_efs\99-45\_SEQUENCE\_ST25.txt  
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Invalid <213> Response:

Use of "Artificial" only as "<213> Organism" response is incomplete,  
per 1.823(b) of New Sequence Rules. Valid response is Artificial Sequence.

Seq#:1,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31,32

Seq#:33,34,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56

Seq#:57,58,59,60,61,62,63,64,65,66,67,68,69,70,71,72,73,74,75,76,77,78,79,80

Seq#:81,82,83,84,85,86,87,88,89,90,91,92,93,94,95,96,97

**VERIFICATION SUMMARY**

DATE: 03/22/2007

PATENT APPLICATION: US/10/031,496E

TIME: 12:58:51

Input Set : N:\efs\03\_22\_07\10031496e\_efs\99-45\_SEQUENCE\_ST25.txt

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